



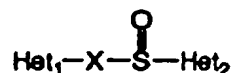
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : C12P 11/00, C07D 401/12	A1	(11) International Publication Number: WO 96/17077 (43) International Publication Date: 6 June 1996 (06.06.96)
(21) International Application Number: PCT/SE95/01416 (22) International Filing Date: 27 November 1995 (27.11.95) (30) Priority Data: 9423968.8                      28 November 1994 (28.11.94)    GB (71) Applicant (for all designated States except US): ASTRA AKTIEBOLAG [SE/SE]; S-151 85 Södertälje (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): GRAHAM, Daniel [GB/GB]; 31 Haslemere Road, Childwall Valley, Liverpool L25 2SE (GB). HOLT, Robert [GB/GB]; 3 Village Way, Kirkby, Fleetham, Northallerton DL7 0TW (GB). LINDBERG, Per [SE/SE]; Gundas gata 40, S-431 51 Mölnådal (SE). TAYLOR, Stephen [GB/GB]; Hillview House, Cleasby, Darlington, Co. Durham DL2 2QY (GB). (74) Agent: ASTRA AKTIEBOLAG; Patent Dept., S-151 85 Södertälje (SE).		(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).  Published With international search report.

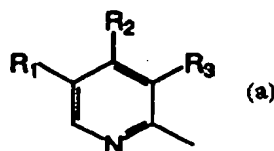
(54) Title: ENANTIOSELECTIVE PREPARATION OF PHARMACEUTICALLY ACTIVE SULFOXIDES BY BIOREDUCTION

## (57) Abstract

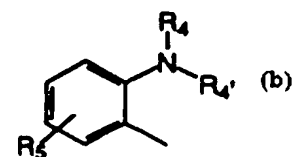
A compound of formula (II), either as a single enantiomer or in an enantiomerically enriched form, wherein Het<sub>1</sub> is (a) or (b), and Het<sub>2</sub> is (c) or (d), and is (e) or (f) (wherein N in the benzimidazole moiety of Het<sub>2</sub> means that one of the carbon atoms substituted by any one of R<sub>6</sub> to R<sub>9</sub> optionally may be exchanged for an unsubstituted nitrogen atom; R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylalkoxy; R<sub>4</sub> and R<sub>4</sub>' are the same or different and selected from hydrogen, alkyl, aralkyl; R<sub>5</sub> is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy; R<sub>6</sub>-R<sub>9</sub> are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkyl-carbonyl, alkoxy-carbonyl, oxazolyl, trifluoroalkyl or adjacent groups R<sub>6</sub>-R<sub>9</sub> may complete together with the carbon atoms to which they are attached optionally substituted ring structures; R<sub>10</sub> is hydrogen or alkoxy-carbonyloxymethyl; R<sub>11</sub> is hydrogen or forms an alkylene chain together with R<sub>3</sub>; R<sub>12</sub> and R<sub>13</sub> are the same or different and selected from hydrogen, halogen or alkyl) is obtained by stereoselective bioreduction of a compound of formula (II) in racemic form.



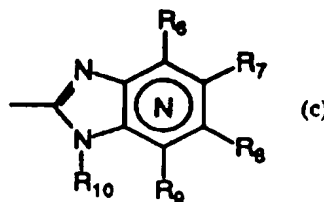
(II)



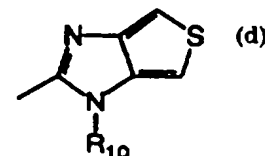
(a)



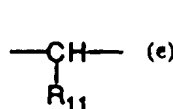
(b)



(c)



(d)



(e)



(f)

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

Enantioselective preparation of pharmaceutically active sulfoxides by bioreduction

5 The present invention relates to a method of obtaining compounds as defined below, either as a single enantiomer or in an enantiomerically enriched form.

### Background to the Invention

10 The racemic form of the compounds prepared by the method of the present invention are known compounds. Some of the compounds are also known in single enantiomeric form. The compounds are active  $H^+K^+ATPase$  inhibitors and they, including their pharmaceutically acceptable salts, are effective acid secretion inhibitors, and known for use as antiulcer agents. The compounds, which include the known compounds omeprazole (compound of formula (IIa) below), lansoprazole  
15 (compound of formula (IIc) below) and pantoprazole (compound of formula (IIb) below), are known for example from European Patent specifications EP 5129 and 124495, EP 174726 and EP 166287.

20 These compounds, being sulfoxides, have an asymmetric centre in the sulfur atom, i.e. exist as two optical isomers (enantiomers). It is desirable to obtain compounds with improved pharmacokinetic and metabolic properties which will give an improved therapeutic profile such as a lower degree of interindividual variation.

25 The separation of enantiomers of omeprazole in analytical scale is described in e.g. J. Chromatography, 532 (1990), 305-19. Also the separation of enantiomers of compounds with which the present invention is concerned, including omeprazole and pantoprazole, is described in German Patent Specification DE 4035455.

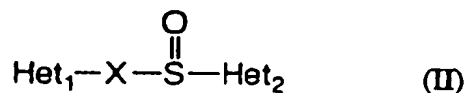
30 Recently there has been a great deal of literature published relating to the synthesis of optically active compounds using biocatalysts. The majority of this work has been aimed at finding routes to single enantiomer forms of pharmaceuticals. The reactions

receiving most attention have been those involved in the preparation of esters, acids and alcohols due to the general utility of these functionalities in synthesis and also because the biocatalysts are readily available.

- 5 Studies on the synthesis of optically active sulfoxides are relatively rare partly due to the small number of pharmaceuticals containing sulfoxide groups and partly due to the fact that enzymes that react with the sulphur centre are not available commercially. The enantioselective reduction of methylphenylsulfoxide to the sulfide has been discussed by Abo M., Tachibana M., Okubo A. and Yamazaki S. (1994)
- 10 Biosci. Biotech. Biochem. 58, 596-597.

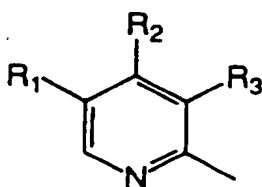
### Description of the Invention

- 15 According to the present invention there is provided a method of obtaining a compound of formula (II) either as a single enantiomer or in an enantiomerically enriched form:

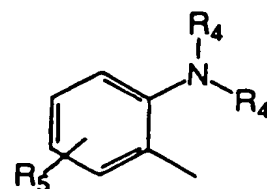


wherein:

Het<sub>1</sub> is

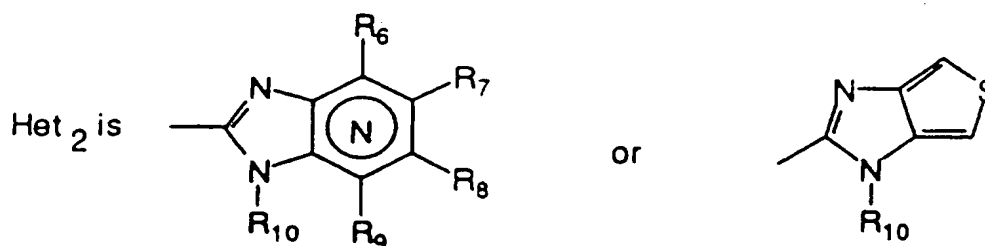


or

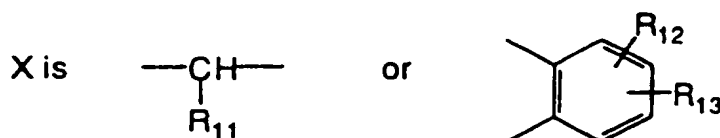


and

20



and



5 wherein:

N in the benzimidazole moiety of Het<sub>2</sub> means that one of the carbon atoms substituted by any one of R<sub>6</sub> to R<sub>9</sub> optionally may be exchanged for an unsubstituted nitrogen atom;

10

R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylalkoxy;

15 R<sub>4</sub> and R<sub>4</sub>' are the same or different and selected from hydrogen, alkyl, aralkyl;

R<sub>5</sub> is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy;

R<sub>6</sub> - R<sub>9</sub> are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxy carbonyl, oxazolyl, trifluoroalkyl or adjacent groups  
 20 R<sub>6</sub> - R<sub>9</sub> may complete together with the carbon atoms to which they are attached optionally substituted ring structures;

$R_{10}$  is hydrogen or alkoxycarbonyloxymethyl;

$R_{11}$  is hydrogen or forms an alkylene chain together with  $R_3$ ;

- 5  $R_{12}$  and  $R_{13}$  are the same or different and selected from hydrogen, halogen or alkyl;

which method comprises stereoselective bioreduction of a compound of formula (II) in racemic form.

- 10 The compounds of formula (II) are active  $H^+K^+$  APTase inhibitors.

The compounds of formula (II) possess a stereogenic (asymmetric) centre which is the sulphur atom which forms the sulfoxide group between the  $Het_1-X-$  and  $Het_2$  moieties. The compounds of formula (II) generally are a racemic mixture initially.

15

In the method according to the present invention the starting compound of formula (II) in racemic form is stereoselectively bioreduced to the corresponding sulfide of the formula:

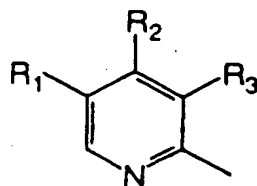


- 20 (wherein  $Het_1$ , X and  $Het_2$  are as defined above). Thus there is obtained compound of formula (II) as a single enantiomer or in enantiomerically enriched form which may be separated from the sulfide produced.

- 25 In the above definitions alkyl groups or moieties may be branched or straight chained or comprise cyclic alkyl groups, for example cycloalkylalkyl.

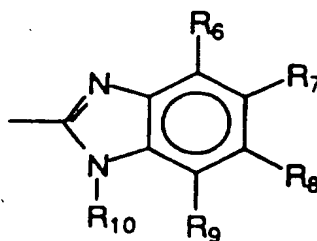
Preferably:

Het<sub>1</sub> is



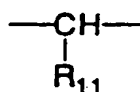
and

Het<sub>2</sub> is



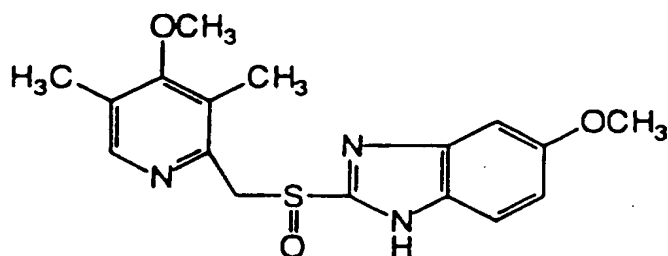
5 and

X is

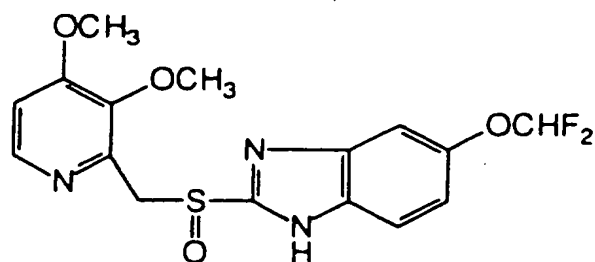


(wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>6</sub> - R<sub>9</sub>, R<sub>10</sub> and R<sub>11</sub> are as defined above).

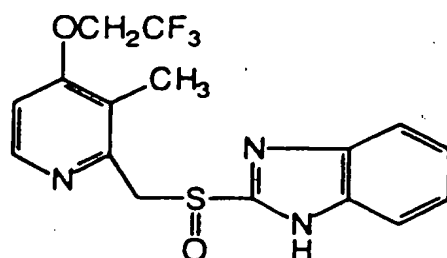
Most preferably the compounds with which the method of the present invention is  
10 concerned are compounds of the formula (IIa) to (IIe):



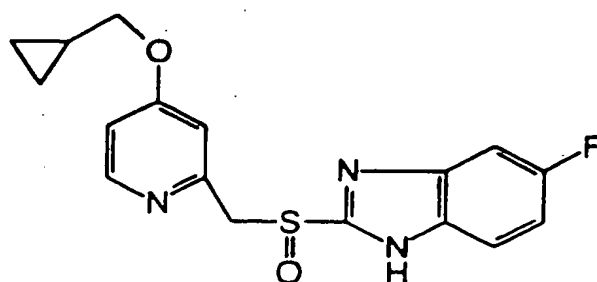
(IIa)



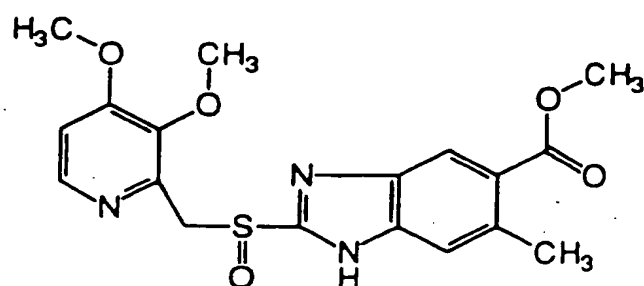
(IIb)



(IIc)



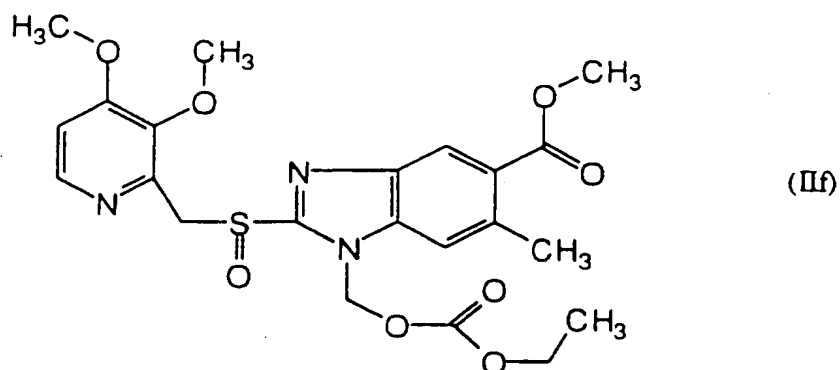
(IIId)



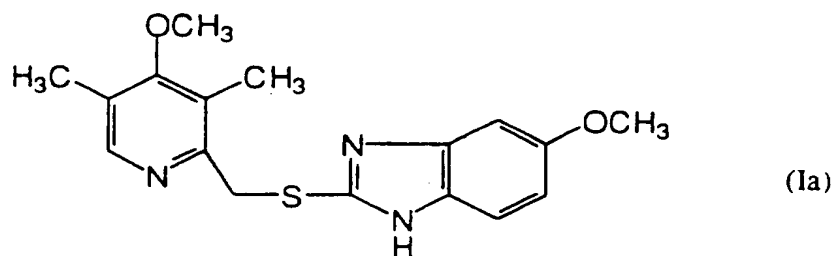
(IIe)

An example of a compound of formula (II) wherein  $\text{R}_{10}$  is alkoxy carbonyloxymethyl is

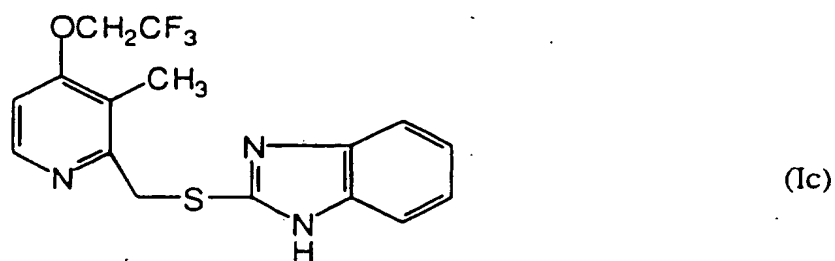
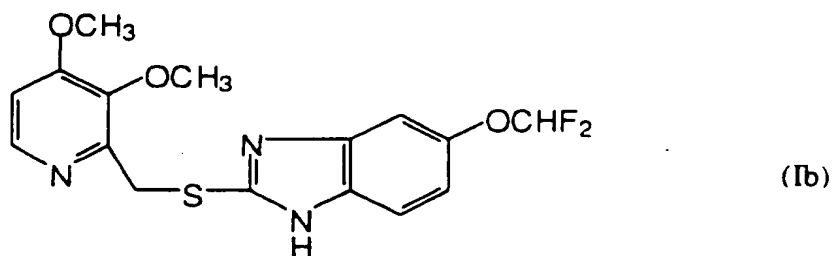


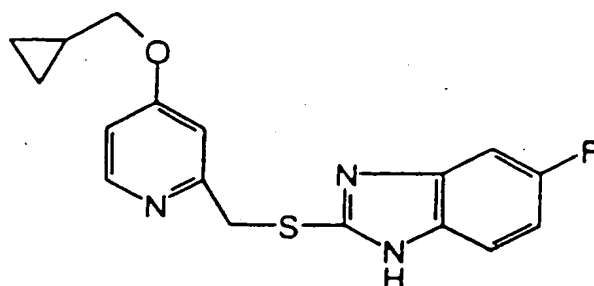


The sulfides formed from the compounds (IIa) - (IIf) will be respectively

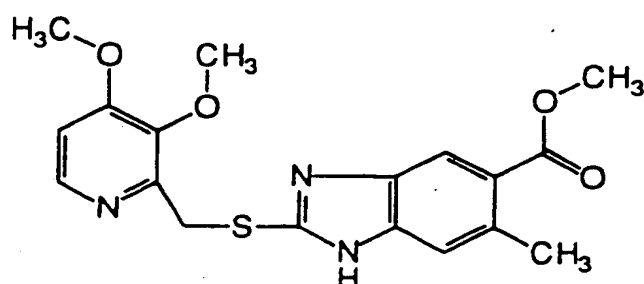


5

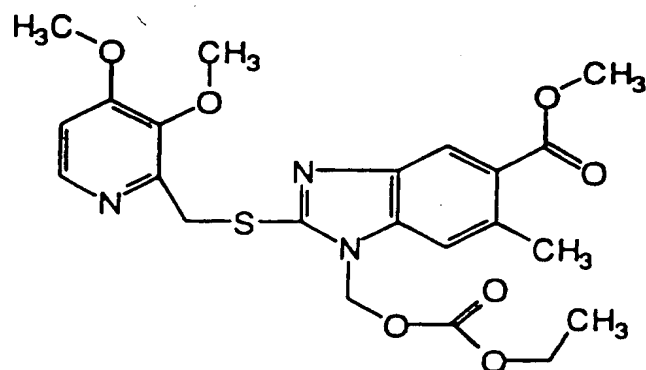




(Id)



(Ic)



(If)

5

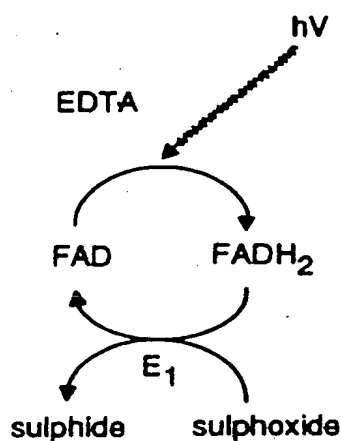
The stereoselective bioreduction according to the present invention may be carried out using a microorganism or an enzyme system derivable therefrom. The organisms used in the method according to the present invention are suitably organisms containing DMSO reductase, for example enterobacteriaceae such as E. Coli and Proteus sp., and purple non-sulfur bacteria of the genus Rhodobacter.

10

Also there may be used DMSO reductase to effect the bioreduction.

- Using DMSO reductase, under anaerobic conditions a cofactor flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) for example may be photo-reduced in the presence of ethylene diamine tetraacetic acid (EDTA). The re-oxidation of the FAD or FMN is coupled to the reduction of the sulfoxide via the DMSO reductase. Hence an artificial biocatalytic cycle is generated in the absence of the cells natural anaerobic electron transport mechanism.

#### Photo catalysed DMSO Reductase Reaction



E<sub>1</sub> - DMSO reductase

10

The compounds of formula (II) are generally acid labile and thus the use of acid conditions is to be avoided. Generally the method according to the invention may be carried out at a pH of 7.6 to 8, suitably about 7.6, and at temperature of 25 to 35°C, suitably about 28°C.

15

According to one embodiment of the invention the method comprises contacting the compound of formula (II) with a microorganism which is:

20

Proteus vulgaris

Proteus mirabilis

Escherichia coli

Rhodobacter capsulatus

The microorganisms used are preferably:

- 5        Proteus vulgaris NCIMB 67  
         Proteus mirabilis NCIMB 8268  
         Escherichia coli ATCC 33694  
         Rhodobacter capsulatus DSM 938

These microorganisms are available from the following culture collections.

10

NCIMB

National Collection Of Industrial And Marine Bacteria  
23 Saint Machar Drive  
Aberdeen AB2 1RY  
15    United Kingdom

ATCC

American Type Culture Collection  
12301 Parklawn Drive  
20    Rockville Maryland 20852  
      United States of America

DSM

Deutsche Sammlung von Mikroorganismen  
25    Mascheroder Weg 1b  
      D-38124  
      Braunschweig  
      Germany

30    The present invention will now be illustrated with reference to the Examples.

EXAMPLE 1

The reductive resolution of the compound of formula (IIa) was investigated using whole cells of E. Coli ATCC 33694, Proteus vulgaris NCIMB 67, Proteus mirabilis NCIMB 8268 and a preparation of DMSO reductase from Rhodobacter Capsulatus DSM 938. E. Coli and the two strains of Proteus were grown under essentially anaerobic conditions in 1 litre screw capped flasks containing 800 ml of medium at 35°C for 48 hours on a rotary shaker. The basal culture medium used had the following composition (g/l): NaH<sub>2</sub>PO<sub>4</sub>, 1.56; K<sub>2</sub>HPO<sub>4</sub>, 1.9; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.8;

MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.2; FeCl<sub>3</sub>, 0.005; Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.001; casamino acids, 1.5. After autoclaving this was supplemented with the following components (g/l): glycerol (for Escherichia coli) or glucose (for Proteus mirabilis), 5; thiamine·HCl, 0.03; nicotinic acid, 0.007. Dimethylsulfoxide (70mM) was added to the medium to serve as terminal electron acceptor for anaerobic respiration and as inducer for dimethylsulfoxide (DMSO) reductase. Trace elements solution (1 ml/litre) was also added to the medium. The stock trace elements solution contained (g/l): CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.02; MnSO<sub>4</sub>·4H<sub>2</sub>O, 0.1; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.1; CaCO<sub>3</sub>, 1.8. E. Coli was also grown anaerobically on the same medium under the same conditions but with 40 mM of fumarate as electron acceptor.

Cells were harvested by centrifuging and washed twice in 10 mM phosphate buffer, pH 7.6. The cells were then resuspended in 100 mM phosphate buffer, pH 7.6 to give a dry cell weight concentration of 3-6 g/l.

50 ml of each cell suspension was then placed in an autotitrator vessel and stirred without aeration in the presence of 0.1-0.3 g/l substrate and glucose (2.5%) at 35°C. The pH was maintained at 7.6 by autotitration with 0.5 mM NaOH.

Cells of Rhodobacter capsulatus DSM 938 were grown and DMSO reductase enzyme prepared as describes in Example 2, below. The reductive resolution of the compound

of formula (IIa) using said enzyme preparation was then carried out as describes in Example 2, except that the compound of formula (IIa) was present at a substrate concentration of 2.9 mM.

## 5 Detection of Products

The bioreduction of the compound of formula (IIa) was followed by reverse phase HPLC on a Spherisorb S5-ODS2 reverse phase column eluted with a 50:50 mixture of acetonitrile and 25 mM sodium phosphate buffer, pH 7.6 at a flow rate of 0.8 ml/min.

10 Under such conditions the compounds of formula (IIa) and (Ia) were well resolved with retention times of 5.2 and 9.8 minutes respectively. Both compounds were detected at a wavelength of 300 nm.

The enantiomeric composition of the compound of formula (IIa) remaining was

15 investigated by the following method. After removal of biomass the aqueous media was extracted with two volumes of ammonia saturated dichloromethane. The pooled organic extracts were dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure to afford a pale brown solid. Then the enantiomeric composition of sulfoxide was determined by HPLC on a Chiralpak AD

20 Column under the following conditions:

	Column	Chiralpak AD 250 mm x 4.6 mm interior diameter with 50 mm guard column
25	Eluent	Hexane:Ethanol:Methanol (40:55:5% V/V)
	Flow	1.0 ml/min
	Injection Volume	20 µl
	Wavelength	300 nm
30	Retention times	
	Compound of formula (Ia)	5.1 min

Compound of formula (IIa):

(+) Enantiomer

8.5 min

(-) Enantiomer

13.4 min

5

The results in Table 1 were obtained for E. Coli ATCC 33694:

TABLE 1

Electron Acceptor	Dry Cell Weight g/l	Time (min)	Compound of formula (IIa) (ppm or mg L <sup>-1</sup> )	Compound of formula (Ia) (ppm or mg L <sup>-1</sup> )	ee (%) (+)	E
Fumarate	6	0	76	0	-	-
		5	65	10	-	-
		15	42	33	-	-
		30	33	42	-	-
		40	30	45	-	-
		65	27	47	-	-
		90	25	50	-	-
		130	22	53	>99	11
DMSO	6	0	74	0	0	-
		15	47	22	28	-
		35	40	34	74	-
		45	34	38	90	20

- 10 In the above Table E is the enantiospecificity constant which may be determined from the extent of conversion and enantiomeric excess of the unreacted compound from the following equation:

$$E = \frac{\ln [(1 - C) \times (1 - ee_s)]}{\ln [(1 - C) \times (1 + ee_s)]}$$

15

where C = conversion

ee<sub>s</sub> = enantiomeric excess of the unreacted compound.

Also in the above table ee is the enantiomeric excess value for the (+) enantiomer of the compound of formula (IIa). The enantiomeric excess value gives an indication of the relative amounts of each enantiomer obtained. The value is the difference between the relative percentages for the two enantiomers. Thus, for example, when the percentage of the (-) enantiomer of the remaining sulphoxide is 97.5% and the percentage for the (+) enantiomer is 2.5%, the enantiomeric excess for the (-) enantiomer is 95%.

At a starting concentration of 0.3 g/l both P. vulgaris NCIMB 67 and P. mirabilis NCIMB 8268 afforded the (+) enantiomer of the compound of formula (IIa) in >99% enantiomeric excess after 48 h (Table 2) in yields of 32% and 5% respectively.

TABLE 2

	P. vulgaris NCIMB 67		P. mirabilis NCIMB 8268	
	Ratio of Enantiomers		Ratio of Enantiomers	
	(+)	(-)	(+)	(-)
Time hours				
0	50	50	50	50
24	79	21	67	33
48	>99	<1	>99	<1

The final concentration of the compound of formula (IIa) after 48 hours was 48 mg/litre for P. vulgaris NCIMB 67 and 8 mg/litre for P. mirabilis NCIMB 8268.



At a lower starting concentration of 0.1 g/l both organisms again afforded the (+) enantiomer of the compound of formula (IIa) in >99% enantiomeric excess, but after 24 hr (Table 3A) in 44% and 40% yields for P. vulgaris NCIMB 67 and P. mirabilis NCIMB 8268 respectively.

5

TABLE 3A

	<u>P. vulgaris</u> NCIMB 67		<u>P. mirabilis</u> NCIMB 8268	
	Ratio of Enantiomers		Ratio of Enantiomers	
	(+)	(-)	(+)	(-)
Time hours				
0	50	50	50	50
5	78	22	79	21
24	>99	<1	>99	<1

The final concentration of the compound for formula (IIa) after 24 hours was 22 mg/litre of P. vulgaris NCIMB 67 and 20 mg/litre for P. mirabilis NCIMB 8268.

- 10 The compound of formula (IIa) acts as a substrate for the isolated DMSO reductase from Rhodobacter capsulatus as shown in Table 3B. The conversion of total sulfoxide after 15 minutes and after 1 hour was 80% and > 90% respectively.

TABLE 3B

	<u>R. capsulatus</u> DSM 938	
	Ratio of Enantiomers	
	(+)	(-)
Time		
0	50	50
15 minutes	50	50
1 h	15	85

## EXAMPLE 2

The reductive resolution of compounds of formula (IIb) and (IIc) was investigated with E. coli ATCC 33694, P. vulgaris NCIMB 67, and a preparation of DMSO reductase from R. capsulatus DSM 938. The reaction conditions were as described in Example 1 for E. coli and P. vulgaris except the compounds of formula (IIb) and (IIc) were present at a substrate concentration of 0.1 g/l. Where a preparation of DMSO reductase was used, the reaction conditions were as follows:

- 10 Cells of R. capsulatus DSM 938 were grown phototrophically in 25 l carboys containing RCV medium supplemented with 50 mM DMSO between two banks of 100 W tungsten bulbs. Cells were harvested by cross-flow filtration followed by centrifugation at 10,000 rpm in a Beckman GSA rotor for 30 min. The pellets were washed once with 50 mM Tris/HCl pH 8.0 and resuspended to approximately 2 g/ml
- 15 in 50 mM Tris/HCl pH 8.0 + 0.5 M sucrose and 1.5 mM EDTA (STE buffer). Lysozyme was added at 0.6 mg/ml and the suspension (approx 1l) was stirred at 30°C for 15 min. After centrifugation as described above the supernatant (periplasmic fraction) was decanted off and brought to 50% saturation with ammonium sulphate. Following centrifugation the supernatant was brought to 70% saturation and re-
- 20 centrifuged. The 50 - 70% pellet was resuspended in a minimum volume of 50 mM Tris/HCl pH 8.0 and dialysed against 3 x 100 volumes of the same buffer being concentrated to approx 5 ml by ultra-filtration through an Amicon PM 10 membrane. The concentrated sample was brought to 150 mM NaCl, charged onto a Sephacryl S200 gel filtration column (column vol = 510 ml) and eluted with 700 ml of 50 mM
- 25 Tris/HCl pH 8.0 + 150 mM NaCl. Peak enzyme activity eluted at approx 320 ml (data not shown) and peak fractions were pooled and concentrated as above.

The "RCV" medium used in this Example had the following composition (g per litre of deionised water or as mM):

30

Propionate

30 mM

	$(\text{NH}_4)_2\text{SO}_4$	1 g
	$\text{KPO}_4$ buffer	10 mM
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	120 mg
	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	75 mg
5	Sodium EDTA	20 mg
	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	24 mg
	Thiamine hydrochloride	1 mg
	Trace element soln.	1 ml

10 Trace element solution (per 250 ml)

	$\text{H}_3\text{BO}_3$	0.7 g
	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	398 mg
	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	188 mg
15	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	60 mg
	$\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$	10 mg

The purified DMSO reductase from Rhodobacter capsulatus DSM 938 was used in the photo-catalysed assay for the reduction of sulfoxide. A 5 ml gas-tight syringe was  
20 filled with approx 3 ml of degassed assay buffer, 50 mM Tris/HCl, 10 mM EDTA, pH 8.0. Flavin mononucleotide (FMN) and sulfoxide were added to the syringe to give final concentrations of 250  $\mu\text{M}$  and 0.3 mM respectively (based on a final volume of 5 ml). The FMN was then reduced via illumination with a tungsten lamp. The DMSO reductase sample (2-10 mg : 2-10  $\mu\text{M}$ ) was then added to the solution and the volume  
25 made up to 5 ml with degassed buffer. The syringe was then illuminated as before. A 1 ml sample was removed immediately ( $t=0$ ) and subsequent sampling repeated over the desired timecourse.

Detection of Products

- The bioreduction of the compounds of formula (IIb) and (IIc) was followed by reverse phase HPLC as described in Example 1 except that the retention times were as follows:

TABLE 4

Compound of Formula	Retention Time (min)
Ib	8.1
IIb	4.2
Ic	10.5
IIc	5.7

- The enantiomeric composition of the compounds of formula (IIb) and (IIc) remaining was investigated by the method of Example 1 except that in the chiral HPLC step, the solvent composition, flow rate and retention time were as follows:

TABLE 5

Compound of Formula	Solvent Composition	Flow Rate (ml/min)	Retention Time (min)
IIb	Hexane/Ethanol (70:30% v/v)	1.0	32.3 (Enantiomer A) 36.6 (Enantiomer B)
IIc	Hexane/Ethanol (70:30% v/v)	0.5	34.3 (Enantiomer A) 36.3 (Enantiomer B)

- The enantiomer which eluted first is referred to as enantiomer A and the second as enantiomer B.

The following results were obtained:

TABLE 6

Biocatalyst	Time (hr)	Ratio of enantiomers of formula (IIb)		Conversion (%)
		A	B	
E coli ATCC 33694	0	50	50	-
	2	45	55	68
	20	40	60	-
	44	<1	>99	89
P vulgaris NCIMB 67	0	50	50	-
	2	53	47	75
	6	53	47	75
	48	53	47	75

Conversion values were for the percentage conversion of the compound of formula (IIb) to the compound of formula (Ib).

5

TABLE 7

Biocatalyst	Time (hr)	Ratio of enantiomers of formula (IIc)		Conversion (%)
		A	B	
E. Coli ATCC 33694	0	50	50	-
	2	39	61	83
	20	21	79	96
P. vulgaris NCIMB 67	0	50	50	-
	2	40	60	45
	6	40	60	54
	48	38	62	85
DMSO reductase (R. capsulatus DSM 938)	0	50	50	-
	0.25	41	59	31
	1.5	41	59	54

Conversion values were for the percentage conversion of the compound of formula (IIc) to compound of formula (Ic).

10

EXAMPLE 3

The reductive resolution of compounds of formula (II<sub>d</sub>) and (II<sub>e</sub>) was investigated.

5

E. coli ATCC 33694 and Proteus mirabilis NCIMB 8268 were screened for the reductive resolution of compounds of formula (II<sub>d</sub>) and (II<sub>e</sub>). Both organisms were grown anaerobically with fumarate as terminal electron acceptor and glycerol (E. coli) or glucose (Proteus mirabilis) as carbon source according to the method of Example 1.

10 After 48 hrs growth at 35°C the cells were harvested by centrifuging at 8k rpm and 4°C and washed by resuspending in 10 mM sodium phosphate buffer, pH 7.6 and centrifuging as above. The cells were finally resuspended in 100 mM sodium phosphate buffer, pH 7.6. The dry cell weights were 8 g/l (E. coli) and 4.5 g/l (Proteus mirabilis) for the compound of formula (II<sub>d</sub>) and 4.3 g/l (E. coli) and 3.9 g/l  
15 (Proteus mirabilis) for the compound of formula (II<sub>e</sub>). The reductive resolution of the compounds of formula (II<sub>d</sub>) and (II<sub>e</sub>) was investigated in an autotitrator containing 40 ml cell suspension, 100 ppm of either substrate and 1 % w/v glucose as energy source.

Detection of Products

20

The bioreduction of the compounds of formula (II<sub>d</sub>) and (II<sub>e</sub>) was followed by reverse phase HPLC as described in Example 1 except that the retention times were as follows:

TABLE 8

Compound of formula	Retention Time (min)
Id	13.7
II <sub>d</sub>	5.0
Ie	9.4
II <sub>e</sub>	4.3

The enantiomeric composition of the compounds of formula (II<sub>d</sub>) and (II<sub>e</sub>) remaining was investigated by the method of Example 1 except that in the chiral HPLC step, the solvent composition, flow rate and retention time were as follows:

5 **TABLE 9**

Compound of Formula	Solvent Composition	Flow Rate (ml/min)	Retention Time (min)
II <sub>d</sub>	Hexane/Ethanol (70:30 % v/v)	1.0	12.9 (Enantiomer A) 21.7 (Enantiomer B)
	Hexane/Ethanol/Methanol (40:55:5 % v/v)	1.0	7.4 (Enantiomer A) 10.6 (Enantiomer B)
II <sub>e</sub>	Hexane/Ethanol (70:30 % v/v)	1.0	26.0 (Enantiomer A) 30.5 (Enantiomer B)

The enantiomer which eluted first is referred to as enantiomer A and the second as enantiomer B.

- 10 The results for the reduction of the compound of formula (II<sub>d</sub>) by both E. coli ATCC 33694 and P. mirabilis NCIMB 8268 were as follows:

**TABLE 10**

Microorganism	Conversion (%)	Enantiomeric excess (%)	Enantiomer	E
E. Coli ATCC 33694	79	88	B	4
P. mirabilis NCIMB 8268	66	90	B	8

- 15 The conversion values were for the percentage conversion of compound of formula (II<sub>d</sub>) to compound of formula (I<sub>d</sub>).

The reduction of the compound of formula (II<sub>e</sub>) by E. coli and P. mirabilis afforded 'B' enantiomer in high enantiomeric excess.

TABLE 11

Microorganism	Conversion (%)	Enantiomeric excess (%)	Enantiomer	E
E. Coli ATCC 33694	78	98.2	8	7
P. mirabilis NCIMB 8268	70	>99	8	11

5

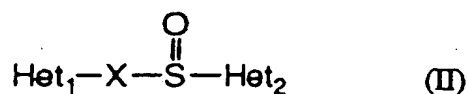
The conversion values were for the percentage conversion of the compound of formula (IIe) to the compound of formula (Ie).

10 These conversion figures were determined from the aqueous dissolved concentration of sulfoxide at the end of the reaction. The magnitude of the enantiospecificity constant is similar to that obtained for the reductive resolution of the compound of formula (IIa) by E. coli ATCC 33694 and Proteus mirabilis NCIMB 8268.



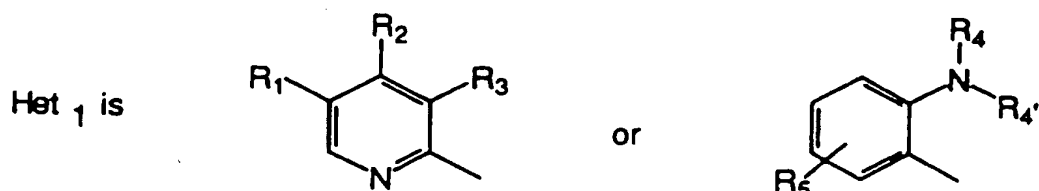
CLAIMS

1. A method of obtaining a compound of formula (II) either as a single enantiomer or in an enantiomerically enriched form:

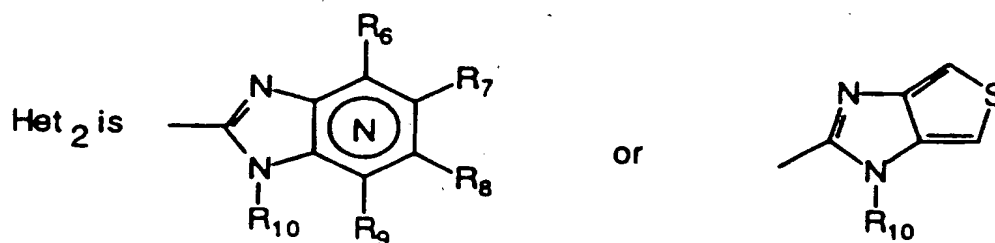


5

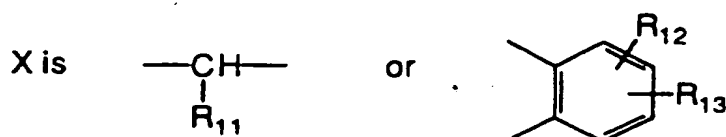
wherein:



and



10 and



wherein:

15 N in the benzimidazole moiety of Het<sub>2</sub> means that one of the carbon atoms substituted by any one of R<sub>6</sub> to R<sub>9</sub>, optionally may be exchanged for an unsubstituted nitrogen atom;

$R_1$ ,  $R_2$  and  $R_3$  are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylalkoxy;

- 5  $R_4$  and  $R_4$  are the same or different and selected from hydrogen, alkyl, aralkyl;

$R_5$  is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy;

- $R_6 - R_9$  are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxy carbonyl, oxazolyl, trifluoroalkyl or adjacent groups
- 10  $R_6 - R_9$  may complete together with the carbon atoms to which they are attached optionally substituted ring structures;

$R_{10}$  is hydrogen or alkoxy carbonyloxymethyl;

15

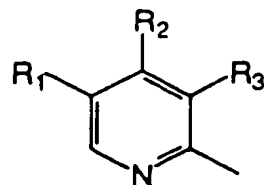
$R_{11}$  is hydrogen or forms an alkylene chain together with  $R_3$ ;

$R_{12}$  and  $R_{13}$  are the same or different and selected from hydrogen, halogen or alkyl;

- 20 which method comprises stereoselective bioreduction of a compound of the formula (II) in racemic form.

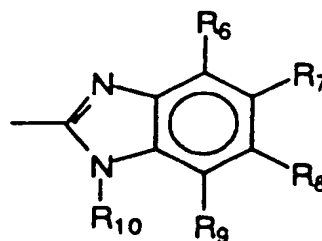
2. A method according to claim 1 wherein:

Het<sub>1</sub> is



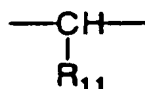
and

Het<sub>2</sub> is



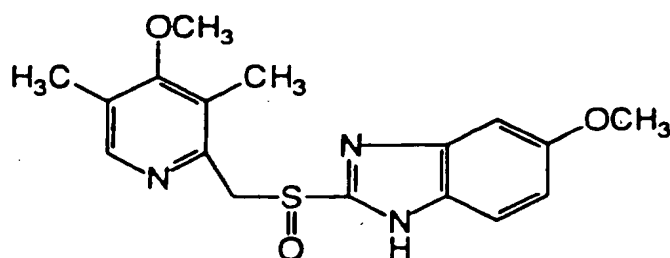
5 and

X is

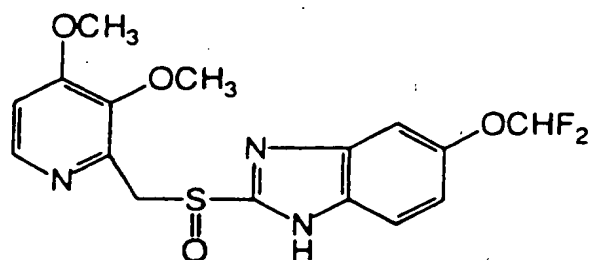


(wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>6</sub> - R<sub>9</sub>, R<sub>10</sub> and R<sub>11</sub> are as defined in claim 1).

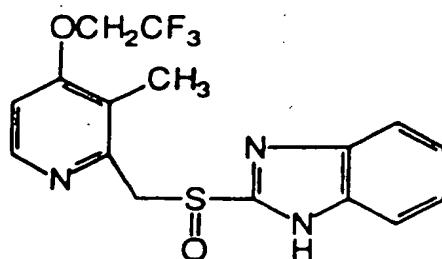
10 3. A method according to claim 1 or 2 wherein the compound of formula (II) is a compound of formula:



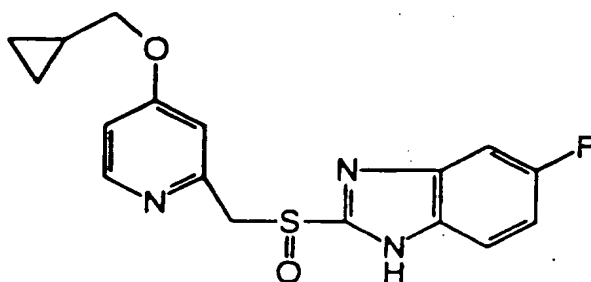
(IIa)



(IIb)

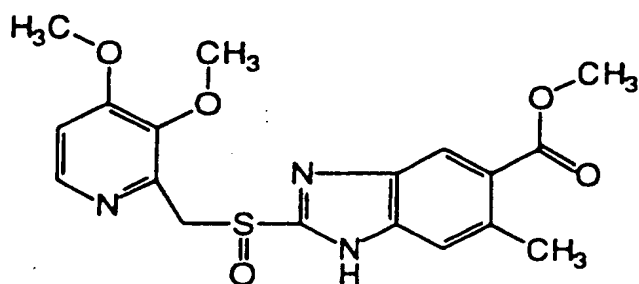


(IIc)



(IIId)

5



(IIe)

4. A method according to any one of the previous claims wherein a single enantiomer of the compound of formula (II) is obtained.

10

5. A method according to claim 3 wherein the compound of formula (II) is the compound of formula (IIa) and the bioreduction is carried out with

Proteus vulgaris

Proteus mirabilis

Escherichia coli

- 5     Rhodobacter capsulatus or  
the DMSO reductase isolated from R. capsulatus.

6. A method according to claim 3 wherein the compound of formula (II) is the compound of formula (IIb) and the bio reduction is carried out with

10

Escherichia coli.

7. A method according to claim 3 wherein the compound of formula (II) is the compound of formula (IIc) and the bio reduction is carried out with

15

Proteus vulgaris

Escherichia coli

Rhodobacter capsulatus or

DMSO reductase isolated from R. capsulatus.

20

8. A method according to claim 3 wherein the compound of formula (II) is the compound of formula (II d) or (II e) and the bio reduction is carried out with

Proteus mirabilis or

25

Escherichia coli.

9. A method according to claim 1 substantially as described in any one of the Examples.

- 30   10. A compound of formula II as defined in claim 1 as a single enantiomer or in an enantiomerically enriched form obtained by the method claimed in any one of claims 1 to 9.

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12P 11/00, C07D 401/12

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, IFIPAT, CA, MEDLINE, EMBASE, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0005129 A1 (AB HÄSSLE), 31 October 1979 (31.10.79) --	1-4
A	Biosci. Biotech. Biochem., Volume 58, No 3, 1994, M. Abo et al., "Enantioselective Reduction of the Sulfoxideto Sulfide in Methyl Phenyl Sulfoxide by Dimethyl Sulfoxide Reductase from Rhodobacter sphaeroides f.s. denitrificans" page 596 - page 597 --	1-10
A	Biochemical Pharmacology, Volume 48, No 2, 1994, E. Kashiya et al., "Chiral Inversion of Drug: Role of Intestinal Bacteria in the Stereoselective Sulphoxide Reduction of Flosequinan" page 237 - page 243 --	1-10

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

\*&amp;\* document member of the same patent family

Date of the actual completion of the international search

19 March 1996

Date of mailing of the international search report

21 -03- 1996

Name and mailing address of the ISA/  
Swedish Patent Office  
Box 5055, S-102 42 STOCKHOLM  
Facsimile No. +46 8 666 02 86

Authorized officer

Gerd Strandell  
Telephone No. +46 8 782 25 00

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

05/02/96

International application No.

PCT/SE 95/01416

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0005129	31/10/79	SE-T3- 0005129	
		AT-B- 374471	25/04/84
		AT-B- 374472	25/04/84
		AT-B- 374473	25/04/84
		AT-B- 375365	25/07/84
		AT-B- 389995	26/02/90
		AU-B,B- 529654	16/06/83
		AU-A- 4602779	18/10/79
		CA-A- 1127158	06/07/82
		CA-A- 1129417	10/08/82
		JP-C- 1312930	28/04/86
		JP-C- 1504537	13/07/89
		JP-A- 54141783	05/11/79
		JP-A- 58192880	10/11/83
		JP-B- 60034956	12/08/85
		JP-B- 63053191	21/10/88
		LU-A- 88307	04/05/94
		SE-A- 7804231	15/10/79
		SU-A,A- 895292	30/12/81
		US-A- 4255431	10/03/81
		US-A- 4337257	29/06/82
		US-A- 4508905	02/04/85